

Practical Problems in Transfusion Medicine

Immunoematology Case Study 1

Part 3: Analysis of Antibody Identification Studies and Conclusions

Observation:

The direct antiglobulin test (DAT) of the patient's cells is positive with polyspecific reagent and with anti-IgG, but is non-reactive with anti-C3. The autocontrol in the panel study is also positive. These positive results demonstrate a mixed field agglutination pattern.

Analysis:

You now know the patient has been recently transfused. The DAT and autocontrol results are consistent and indicate that IgG has been bound *in vivo* to some, but not all of the cells in the patient's circulation. This result confirms the IgG is not an autoantibody; it must be alloantibody bound to transfused red cells.

Observation:

The reaction of the patient's cells with anti-A displays a mixed field pattern of agglutination.

Analysis:

Since some circulating cells are non-reactive with anti-A, the patient was most likely transfused with Group O donor cells.

Observation:

The patient's cells are non-reactive with anti-K; they are strongly reactive with anti-C and with anti-e. Tests with anti-E and with anti-c are also positive, but with mixed field agglutination .

Analysis:

The patient's cells are phenotype C positive, e positive, K negative. However, reliable interpretation of the typing tests with anti-E and anti-c cannot be made. The non-reactive population of cells may belong to the patient or they may be donor red cells.

Observation:

The patient's serum reacts in the antihuman globulin (AHG) phase of the panel study with 8 out of 10 reagent cells. No reactivity is seen in the room temperature or 37°C incubation phases of testing.

Analysis:

All serum antibody activity is IgG. The presence of Lewis, MN, and P₁ antibodies can be ruled out because they are IgM antibodies that typically react at ambient or colder temperatures.

Panel cells #1 and #10 are non-reactive in the AHG phase. Therefore, antibody directed toward any antigen expressed on either of these panel cells cannot be present in the patient's serum. The remaining antibody specificities that cannot be ruled out are E, K, Jk^b, Fy^a, and S, since these antigens are missing from both cells.

cells	Rh-hr					Kell		Kidd		Duffy		Lewis		MNSs				P	RT	37°C	(Anti-IgG) AHG	
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Le ^a	Le ^b	M	N	S	s	P ₁				
1	+	+	0	0	+	0	+	+	0	0	+	0	0	+	0	+	+	+	+	0	0	0
10	+	0	0	+	+	0	+	+	0	0	0	+	0	+	+	0	+	0	0	0	0	0

To prove or disprove the presence of anti-E, -K, -Jk^b, -Fy^a, or -S individually, the patient's serum must be tested with a cell that is antigen-positive for one possibility and antigen-negative for all the others. The presence of anti-Jk^b is therefore proven. Cell #9 is reactive with the patient's serum and types Jk(b+); it is also E negative, K negative, Fy(a-), and S negative so none of the other antibody possibilities could be responsible for this reaction. Since the patient's serum reacts with two Jk(b-) cells (# 7 and # 8), at least one other antibody must be present.

cells	Rh-hr					Kell		Kidd		Duffy		Lewis		MNSs				P	RT	37°C	(Anti-IgG) AHG	
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Le ^a	Le ^b	M	N	S	s	P ₁				
7	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	+	+	+	+	0	0	2+
8	0	0	0	+	+	0	+	+	0	+	+	0	+	0	+	+	+	+	+	0	0	2+
9	+	0	0	+	+	0	+	+	+	0	+	0	0	+	0	0	+	+	+	0	0	2+

In order to prove or disprove the presence of the remaining antibody possibilities, additional cells of selected blood-group phenotype need to be tested with the patient's serum. Also, an eluate should be prepared from the patient's cells and tested for the presence of any alloantibody that may have bound to donor cells and not be present in the patient's serum. Additional phenotyping of the patient's sample may also be helpful.

Your laboratory does not have the resources to perform these additional tests so your supervisor arranges to send the patient's sample to the nearest immunohematology reference laboratory, which is located at the regional blood center 50 miles away.

Communication with the Intensive Care Unit (ICU)

The ICU calls to report the patient's condition has worsened and the need for transfusion is now more urgent. Your supervisor explains the status of the investigation to the physician:

- Current tests indicate anti-Jk^b is present in the patient's serum.
- The out-of-state hospital where the patient had been transfused last month reports that they identified anti-Jk^b and anti-Fy^a. They transfused her with two Group O Jk(b-) Fy(a-) units, which were well-tolerated.
- Other antibodies may also be present, but have not yet been identified. More information will likely not be available for at least six hours.
- Anti-Jk^b, anti-Fy^a, and the other candidate specificities are known to be clinically significant and can potentially cause hemolysis of antigen-positive donor cells.

They discuss the most appropriate transfusion to give if the patient is in critical need of blood before the problem is resolved. Although your transfusion service cannot perform extended phenotyping, the patient's serum reacts strongly and would likely detect donor units positive for the antigen(s) against which demonstrable patient antibody is directed. Even if all five antibodies are present, one compatible unit should be found out of every 35 donors. It is possible that some crossmatch-compatible blood can be found among the hospital Group A and Group O donor inventory.

The risk involved is the possibility that crossmatch will not detect incompatible antigen that is weakly expressed on donor cells. Some weak antibodies may not detect antigen that is expressed in single dose; i.e. expression that is derived from a heterozygous genotype. However, crossmatch-compatible blood in the face of this serological situation is relatively safe and should be given without hesitation if the patient is in critical need.

Outcome

Your supervisor directs you to crossmatch the patient with all A and O donors in inventory. After testing 25 units you find one that is negative by AHG. You notify the Emergency Department. The physician authorizes release of that unit for transfusion. It is infused slowly and the patient is observed. She tolerates the unit with no ill effects.

Reference Laboratory Investigation:

ABO and Rh Typing

Tests of cells with:

Anti-A	Anti-B	Anti-D
2+ mf	0	3+

Tests of serum with:

A ₁ Cells	B Cells
0	3+

Direct Antiglobulin Test (DAT)

Tests of cells with:

Polyspecific AHG	Anti-IgG	Anti-C3
1+ mf	1+ mf	0

Panel Study: LIS added

cells	Rh-hr					Kell		Kidd		Duffy		Lewis		MNSs				P	RT	37°C	(Anti-IgG) AHG		
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Le ^a	Le ^b	M	N	S	s	P ₁					
1	+	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0
2	+	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	2+
3	+	0	+	+	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	0	0	3+
4	+	0	0	+	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	0	0	2+
5	0	+	0	+	+	0	+	+	0	0	+	0	+	+	+	+	+	+	+	+	0	0	2+
6	0	0	+	+	+	0	+	+	+	+	+	0	+	+	+	+	+	+	+	+	0	0	2+
7	0	0	0	+	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	0	0	0
8	0	0	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	0	2+
9	0	0	0	+	+	0	+	+	0	0	+	+	0	+	0	0	0	+	0	0	0	0	0
10	0	0	0	+	+	0	+	+	+	+	0	0	0	+	0	+	+	+	+	+	0	0	2+
auto																					0	0	1+mf

The panel study reveals IgG antibody activity only and a weakly positive autocontrol with mixed field agglutination. Three panel cells (#1, #7, #9) are non-reactive, allowing a ruleout

of all common IgG antibodies except anti-E, -Jk^b, -Fy^a, and -S. Although not ruled out by the hospital panel, here cells #1 and #7 are K positive and non-reactive with the patient's serum, allowing anti-K to be ruled out.

This panel cannot confirm the presence of anti-Jk^b or of anti-Fy^a, but it can demonstrate the presence of anti-S. Reactive cells #4 and #5 are E negative, Jk(b-), Fy(a-), but are S positive. The especially strong reaction with E positive cell #3 raises suspicion that anti-E is present. (Since this cell lacks alternative antigen e, it expresses E antigen more strongly than cell #6, which is positive for both E and e.)

A panel of enzyme-treated cells is then tested to isolate activity of anti-Jk^b, seen by the hospital, and of a possible anti-E.

HELPFUL FACTS – Dosage Effect

The strength with which red cell antigens are expressed varies from person to person depending on their individual blood group genotype. People who are homozygous for a particular blood group allele carry a double dose of that antigen on their red cells and express it more strongly than people who are heterozygous for that allele and carry only a single dose of antigen.

The following comparison should help explain the dosage effect:

	Kidd Genotype	Jk ^a Phenotype	Jk ^a Antigen Dosage	Jk ^a Antigen Expression
Person #1	Jk ^a /Jk ^a	Jk(a+)	Double dose	Strong
Person #2	Jk ^a /Jk ^b	Jk(a+)	Single dose	Less strong

Both persons are Jk(a+). However, every copy of Kidd protein on the red cells of Person #1 carries the Jk^a antigen. Only some copies of Kidd protein on the red cells of Person #2 carry Jk^a antigen; the others carry Jk^b. Therefore, Jk^a is present in a double dose and expressed more strongly in Person #1.

The dosage effect is important to consider when interpreting antibody identification studies. Some weak antibodies may not react with cells that are indeed antigen positive, but carry only a single dose.

Example of weak anti-Jk^a exhibiting dosage effect:

cells	Rh-hr					Kell		Kidd		Duffy		Lewis		MNSs				P	(Anti-IgG) AHG	
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Le ^a	Le ^b	M	N	S	s	P ₁		
1	+	+	0	0	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	1+
2	+	+	0	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	+	0
3	+	0	+	+	0	0	+	+	+	+	+	+	0	0	+	+	+	+	0	0
4	+	0	+	+	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0
5	+	0	+	+	+	0	+	+	+	0	+	0	+	0	+	0	+	+	0	0
6	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0
7	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	+	+	+	+	1+
8	0	0	0	+	+	0	+	+	0	+	+	0	+	0	+	+	+	+	+	1+

HELPFUL FACTS

Enzyme-treated Reagent Cells

Enzyme treatment **enhances** cell expression of **Rh** and **Kidd** blood group antigens. Serologic activity of antibodies to these antigens is strengthened in tests with enzyme-treated panel cells.

Enzyme treatment **inactivates** antigens of the **MNS** and **Duffy** blood groups. Activity of antibodies to these antigens cannot be detected in tests with enzyme-treated panel cells.

Expression of **Kell** blood group antigens is generally **unaffected** by enzyme treatment.

Enzyme-treated reagent cells are most commonly treated with ficin. Other enzymes, as well as reducing agents, are used as cell treatments in advanced studies to further differentiate antibody specificity.

Panel Study: Enzyme-treated cells

cells	Rh-hr					Kell		Kidd		Duffy		Lewis		MNSs				P	37°C	(Anti-IgG) AHG	
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Le ^a	Le ^b	M	N	S	s	P ₁			
1	+	+	0	0	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	0
2	+	+	0	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	+	3+	3+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	0	+	+	+	+	0	3+	3+
4	+	0	+	+	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	3+	3+
5	+	0	+	+	+	0	+	+	0	0	+	0	+	0	+	0	+	0	0	3+	3+
6	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0	0	3+	3+
7	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	+	+	+	+	0	0
8	0	0	0	+	+	0	+	+	0	+	+	0	+	0	+	+	+	+	+	0	0
9	+	0	0	+	+	0	+	+	+	0	+	0	0	+	0	0	+	+	+	3+	3+
10	+	0	0	+	+	0	+	+	+	0	0	+	0	+	+	0	+	0	0	3+	3+

This panel study is non-informative for anti-Fy^a or anti-S, since these antibodies do not react with enzyme-treated cells.

The results of this panel confirm the presence of anti-E and anti-Jk^b. Cells #3 and #5 are E positive and Jk(b-); they react with the patient's serum, confirming the presence of anti-E. Cells #2, #6, #9, and #10 are Jk(b+) and E negative; they also react with the patient's serum, confirming the presence of anti-Jk^b. All cells that are E+ and/or Jk(b+) react with the patient's serum and all cells negative for both E and Jk^b (#1, #7, #8) are non-reactive.

An additional panel of cells selected for specific phenotypes to support the presence of antibodies detected so far and to investigate the possibility of anti-Fy^a was then tested with the patient's serum:

Panel Study: Selected cells, LIS added

cells	Rh-hr					Kell		Kidd		Duffy		Lewis		MNSs				P	37°C	(Anti-IgG)	
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Le ^a	Le ^b	M	N	S	s	P ₁		AHG	
1	+	+	0	0	+	0	+	+	0	0	+	+	0	0	+	+	+	+	+	0	2+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	+	+	0	+	+	+	0	2+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	0	0	3+
4	+	0	+	+	0	0	+	+	0	0	+	0	0	+	0	0	+	+	+	0	3+
5	+	0	0	+	+	0	+	+	0	0	0	0	+	0	+	0	+	0	0	0	0
6	0	0	0	+	+	0	+	+	0	0	+	+	0	+	0	0	+	0	0	0	0
7	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	0	+	+	+	0	2+
8	0	0	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	0	2+
9	+	0	0	+	+	0	+	+	0	0	+	0	0	+	0	0	+	+	+	0	0
10	+	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	0	2+

Three cells (#5, #6, and #9) are E-, Jk(b-), Fy(a-), and S-, and are non-reactive with the patient's serum.

Two cells (#7 and #8) are Fy(a+), and are also E negative, Jk(b-), and S negative. They both react with the patient's serum, confirming the presence of anti-Fy^a. The other reactive cells are positive for at least one of the antigens against which the patient's four identified antibodies are directed.

Panel Study – Eluate prepared from patient cells

cells	Rh-hr					Kell		Kidd		Duffy		Lewis		MNSs				P	(Anti-IgG)	Wash control*	
	D	C	E	c	e	K	k	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Le ^a	Le ^b	M	N	S	s	P ₁	AHG		
1	+	+	0	0	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	0
2	+	+	0	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	+	0	0
3	+	0	+	+	0	0	+	+	+	+	+	+	0	0	+	+	+	+	0	3+	0
4	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	+	+	3+	0
5	+	0	+	+	+	0	+	+	+	0	+	0	+	0	+	0	+	0	0	3+	0
6	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0	0	3+	0
7	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	+	+	+	+	3+	0
8	0	0	0	+	+	0	+	+	0	+	+	0	+	0	+	+	+	+	+	3+	0
9	+	0	0	+	+	0	+	+	+	0	+	0	0	+	0	0	+	+	+	0	0
10	+	0	0	+	+	0	+	+	0	0	0	+	0	+	+	0	+	0	0	0	0

*tests of supernatant from last wash of cells

The four non-reactive cells (#1, #2, #9, #10) allow ruleout of all common IgG antibodies, except anti-E and anti-S. Cell #5 is E positive and S negative, confirming the presence of anti-E. Cells #6, #7, and #8 are S positive and E negative, confirming anti-S.

Phenotyping Tests

Cells tested	Tests of cells with:										
	Anti-C	E	c	e	K	Jk ^a	Jk ^b	Fy ^a	Fy ^b	S	s
Patient	3+	1+mf	3+mf	3+	0	3+	0	0	3+	1+mf	3+
Ag pos control	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

The patient's blood sample types K⁻, Jk(b⁻), Fy(a⁻), indicating both her own red cells and those cells surviving from her recent transfusions carry this phenotype. This is consistent with the out-of-state hospital's report of providing her with Jk(b⁻), Fy(a⁻) red cell transfusions. The mixed field typing results are difficult to interpret, but since she has made both alloanti-E and alloanti-S, she must be negative for those antigens. The observation that the patient's anti-E and anti-S can be eluted from her circulating cells indicates that donor cells must be positive for those antigens.

HELPFUL FACTS – Calculation of Phenotype Frequency

The expected frequency of a particular blood group phenotype can be calculated by multiplying the individual frequencies for the negative phenotype of each antigen.

In this case study the patient requires donor blood with the phenotype E negative, Jk(b⁻), Fy(a⁻), S negative. The frequencies in the random donor population for the negative phenotype of each individual antigen are:

Phenotype	Frequency
E negative	0.71
Jk(b ⁻)	0.28
Fy(a ⁻)	0.34
S negative	0.45

$$0.71 \times 0.28 \times 0.34 \times 0.45 = 0.03$$

100 units of blood tested should provide 3 of the appropriate phenotype for this patient. Approximately one out of every 34 random units will be of the appropriate phenotype.

For practical purposes in the transfusion service, estimating frequencies with "ballpark figures" is faster and equally useful:

Phenotype	Frequency
E negative	3 out of 4
Jk(b ⁻)	1 out of 4
Fy(a ⁻)	1 out of 3
S negative	1 out of 2

4 units are needed to find one Jk(b⁻) donor

3 x 4 = 12 units are needed to find one Jk(b⁻) Fy(a⁻) donor

2 x 12 = 24 units are needed to find one Jk(b⁻) Fy(a⁻) S negative donor

25% (or 6) of them will be E positive

At least 30 units are needed to find one Jk(b⁻) Fy(a⁻) S negative E negative donor

How many units could you expect to find for this patient in your hospital inventory?

Conclusions

The patient's serum contains anti-E, anti-Jk^b, anti-Fy^a, and anti-S. The anti-Jk^b and anti-Fy^a had been identified previously. Recent transfusion of E positive and S positive donor cells likely provoked the anti-E and anti-S that are now detectable. Some of these cells are surviving in the patient's circulation and patient antibody can be eluted from them. The regional blood center will test for E negative, Jk(b-), Fy(a-), and S negative donors to support the patient's transfusion needs.

Learning Objectives

Study of Immunohematology Case Study 1 should allow achievement of the following objectives:

- 1. To recognize the serological difference between a positive DAT reaction caused by an IgG autoantibody and one caused by a patient's IgG alloantibody reactive with circulating donor cells*
- 2. To recognize a mixed field pattern of agglutination and explain its cause*
- 3. To discuss the importance of knowing a patient's recent transfusion history, especially in the presence of a positive DAT*